

Amendments to the Specification:

Please replace the paragraph beginning at page 3, line 1, with the following rewritten paragraph:

-- from amino acid 1 to between amino-acids 1027 and 1062 of SEQ ID NO:20 ~~SEQ ID N°20~~ for $\alpha_2\delta$ -2,
D1 - from amino acid 1 to between amino-acids 984 and 1019 of SEQ ID NO:22 ~~SEQ ID N°22~~ for $\alpha_2\delta$ -3. --

Please replace the paragraph beginning at page 3, line 6, with the following rewritten paragraph:

3) A purified or isolated nucleic acid according to 1), having at least 90% identity with the sequence encoding:
D2 - from amino acid 1 to between amino-acids 1047 and 1062 of SEQ ID NO:20 ~~SEQ ID N°20~~ for $\alpha_2\delta$ -2,
- from amino acid 1 to between amino-acids 1004 and 1019 of SEQ ID NO:22 ~~SEQ ID N°22~~ for $\alpha_2\delta$ -3. --

Please replace the paragraph beginning at page 3, line 9, with the following rewritten paragraph:

4) A purified or isolated nucleotide sequence according to 1) wherein said sequence is the sequence of SEQ ID NO:1, ~~SEQ ID N°1~~, SEQ ID NO:2, ~~SEQ ID N°2~~, SEQ ID NO:3, ~~SEQ ID N°3~~, SEQ ID NO:7, ~~SEQ ID N°7~~, SEQ ID NO:8, ~~SEQ ID N°8~~, SEQ ID NO:9, ~~SEQ ID N°9~~, SEQ ID NO:13, ~~SEQ ID N°13~~, SEQ ID NO:14, ~~SEQ ID N°14~~, SEQ ID NO:15, ~~SEQ ID N°15~~, SEQ ID NO:19, ~~SEQ ID N°19~~, or SEQ ID NO:21, ~~SEQ ID N°21~~. --
D3

Please replace the paragraph beginning at page 3, line 13, with the following rewritten paragraph:

5) A purified or isolated nucleic acid, having at least 90% identity with the nucleotide sequence of SEQ ID NO:19 ~~SEQ ID N°19~~ or SEQ ID NO:21, ~~SEQ ID N°21~~. --
D4

Please replace the paragraph beginning at page 3, line 16, with the following rewritten paragraph:

D⁵

-- 6) A purified or isolated polynucleotide comprising at least 10 consecutive nucleotides of the nucleotide sequence of SEQ ID NO:19 SEQ ID N°19 or SEQ ID NO:21. SEQ ID N°21. --

Please replace the paragraph beginning at page 3, line 19, with the following rewritten paragraph:

D⁶

-- 7) A polynucleotide probe or primer hybridizing, under stringent conditions, with the with the nucleotide sequence of SEQ ID NO:19 SEQ ID N°19 or SEQ ID NO:21. SEQ ID N°21. --

Please replace the paragraph beginning at page 4, line 24, with the following rewritten paragraph:

D⁷

-- 14) A recombinant polypeptide according to 13), having at least 80% amino-acid identity with a polypeptide comprising:
- from amino acid 1 to between amino acids 1027 and 1062 of the amino acid sequence of SEQ ID NO:20, SEQ ID N°20, or
- from amino acid 1 to between amino acids 1019 and 1079 of the amino acid sequence of SEQ ID NO:22. SEQ ID N°22. --

Please replace the paragraph beginning at page 4, line 31, with the following rewritten paragraph:

D⁸

-- 15) A recombinant polypeptide according to 14), wherein said recombinant polypeptide is selected from the group consisting of the amino acid sequences of SEQ ID NO:4, SEQ ID n°4, SEQ ID NO:5, SEQ ID n°5, SEQ ID NO:6, SEQ ID n°6, SEQ ID NO:10, SEQ ID n°10, SEQ ID NO:11, SEQ ID n°11, SEQ ID NO:12, SEQ ID n°12, SEQ ID NO:16, SEQ ID n°16, SEQ ID NO:17 SEQ ID n°17 and SEQ ID NO:18. SEQ ID n°18. --

Please replace the paragraph beginning at page 5, line 17, with the following rewritten paragraph:

D⁹ -- 20) A method according to 16), wherein said secreted soluble recombinant calcium channel $\alpha_2\delta$ -n subunit polypeptide is selected from polypeptides having at least 80%, preferably 90%, more preferably 95%, and most preferably 98 or 99% amino-acid identity with the polypeptide comprising from amino acid 1 to between amino-acids 984 and 1063, preferably between amino-acids 994 and 1054, and most preferably between amino-acids 1019 and 1054 of SEQ ID NO:5 ~~SEQ ID N°5~~ or SEQ ID NO:16 ~~SEQ ID N°16~~. --

Please replace the paragraph beginning at page 5, line 24, with the following rewritten paragraph:

D¹⁰ -- 21) A method according to 16), wherein said secreted soluble recombinant calcium channel $\alpha_2\delta$ -n subunit polypeptide is selected from the group consisting of SEQ ID NO:6 ~~SEQ ID N°6~~, 7, 8, 9, 13, 14 and 15, with the polypeptides of SEQ ID NO:9 ~~SEQ ID N°9~~ and SEQ ID NO:15 ~~SEQ ID N°15~~ being most preferred. --

Please replace the paragraph beginning at page 8, line 31, with the following rewritten paragraph:

D¹¹ -- The inventors believe that the soluble secreted $\alpha_2\delta$ -2 subunit polypeptides which are as close as possible to the native sequence and which are therefore more likely to retain their native folding and hence their [³H]gabapentin binding properties are those corresponding to the native protein in which amino-acid stretch __1027__ to __the C-terminal end__ of the amino-acid sequence of SEQ ID NO:20 ~~SEQ ID N°20~~ has been deleted. The skilled scientist can quite easily determine within this amino-acid stretch the optimal $\alpha_2\delta$ -2 subunit polypeptides. --

Please replace the paragraph beginning at page 9, line 1, with the following rewritten paragraph:

D¹² -- their native folding and hence their ~~thir~~ [³H]gabapentin binding properties are those corresponding to the native protein in which amino-acid stretch __984__ to __C-terminal

end ___ of the amino-acid sequence of SEQ ID NO:22 ~~SEQ ID N°22~~ has been deleted.

D12 The skilled scientist can quite easily determine within this amino-acid stretch the optimal $\alpha_2\delta$ -3 subunit polypeptides. --

Please replace the paragraph beginning at page 9, line 7, with the following rewritten paragraph:

-- The invention therefore particularly concerns a nucleotide sequence encoding a polypeptide having at least 80% identity with the polypeptide comprising from amino-acid 1 to between amino-acids _1027_ and _1145_, preferably to between amino-acids _1062_ and _1145_ of SEQ ID NO:20. ~~SEQ ID N°20~~.

D13 Preferred nucleotide sequences include those of SEQ ID NO:1, ~~SEQ ID N°1~~, SEQ ID NO:2 ~~SEQ ID N°2~~ and SEQ ID NO:3. ~~SEQ ID N°3~~. --

Please replace the paragraph beginning at page 9, line 14, with the following rewritten paragraph:

-- The invention also concerns a nucleotide sequence encoding a polypeptide having at least 80% identity with the polypeptide comprising from amino-acid 1 to between amino-acids _984_ and 1085_, preferably to between ~~to between~~ amino-acids 1019_ and _1085_ of SEQ ID NO:22. ~~SEQ ID N°22~~.

D14 Preferred nucleotide sequences include those of SEQ ID NO:7, ~~SEQ ID N°7~~, SEQ ID NO:8 ~~SEQ ID N°8~~ and SEQ ID NO:9. ~~SEQ ID N°9~~. --

Please replace the paragraph beginning at page 9, line 35, with the following rewritten paragraph:

D15 -- In a first preferred embodiment of the above method, the nucleic acid encodes a secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit polypeptide of SEQ ID NO:4, ~~SEQ ID N°4~~, SEQ ID NO:5, ~~SEQ ID N°5~~, SEQ ID NO:6, ~~SEQ ID N°6~~, SEQ ID NO:10, ~~SEQ ID N°10~~, SEQ ID NO:11, ~~SEQ ID N°11~~, SEQ ID NO:12, ~~SEQ ID N°12~~, SEQ ID NO:16, ~~SEQ ID N°16~~, SEQ ID NO:17 ~~SEQ ID N°17~~ and SEQ ID NO:18. ~~SEQ ID N°18~~. --

Please replace the paragraph beginning at page 10, line 10, with the following rewritten paragraph:

--The present invention also encompasses a family of recombinant vectors comprising any one of the nucleic acids described herein. Firstly, the invention deals with a recombinant vector comprising a nucleic acid selected from the group consisting of:

(a) a purified or isolated nucleic acid encoding a α -secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit having at least 80% amino acid identity with the polypeptide of SEQ ID NO:20 ~~SEQ ID N°20~~ or 22, or a sequence complementary thereto;

D⁶ (b) a purified or isolated nucleic acid having at least 90% nucleotide identity with a polynucleotide selected from the group consisting of the nucleotide sequences of SEQ ID NO:1, SEQ ID N°1, SEQ ID NO:2, SEQ ID N°2, SEQ ID NO:3, SEQ ID N°3, SEQ ID NO:7, SEQ ID N°7, SEQ ID NO:8, SEQ ID N°8, SEQ ID NO:9, SEQ ID N°9, SEQ ID NO:13, SEQ ID N°13, SEQ ID NO:14, SEQ ID N°14, SEQ ID NO:15 SEQ ID N°15 or a sequence complementary thereto;

(c) a purified or isolated polynucleotide comprising at least 10 consecutive nucleotides of a nucleic acid described in (a) or (b) or a sequence complementary thereto.--

Please replace the paragraph beginning at page 10, line 26, with the following rewritten paragraph:

D¹⁷ --Recombinant expression vectors comprising a nucleic acid encoding secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit polypeptides that are described in the present specification are also part of the invention. These include, but are not restricted to, nucleic acids encoding from amino-acid 1 to between amino-acids 1027 and 1145, preferably between amino-acids 1062 and 1145 of SEQ ID NO:20, ~~SEQ ID N°20~~, as well as nucleic acids encoding from amino-acid 1 to between amino-acids 984 and 1085, preferably between amino-acids 1019 and 1085, of SEQ ID NO:22. ~~SEQ ID N°22~~.--

Please replace the paragraph beginning at page 10, line 33, with the following rewritten paragraph:

D18 -- Another preferred embodiment of the recombinant vectors according to the invention consist of expression vectors comprising a nucleic acid encoding $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit polypeptides of the invention, and more preferably a nucleic acid encoding a polypeptide selected from the group consisting of the amino acid sequences of SEQ ID NO:4, ~~SEQ ID N°4~~, SEQ ID NO:5, ~~SEQ ID N°5~~, SEQ ID NO:6, ~~SEQ ID N°6~~, SEQ ID NO:10, ~~SEQ ID N°10~~, SEQ ID NO:11, ~~SEQ ID N°11~~, SEQ ID NO:12, ~~SEQ ID N°12~~, SEQ ID NO:16, ~~SEQ ID N°16~~, SEQ ID NO:17 ~~SEQ ID N°17~~ and SEQ ID NO:18. ~~SEQ ID N°18~~. --

Please replace the paragraph beginning at page 13, line 7, with the following rewritten paragraph:

D19 -- The present invention also concerns a method for producing one of the amino acid sequences described herein and especially a polypeptide selected from the group consisting of the amino acid sequences of SEQ ID NO:4, ~~SEQ ID N°4~~, SEQ ID NO:5, ~~SEQ ID N°5~~, SEQ ID NO:6, ~~SEQ ID N°6~~, SEQ ID NO:10, ~~SEQ ID N°10~~, SEQ ID NO:11, ~~SEQ ID N°11~~, SEQ ID NO:12, ~~SEQ ID N°12~~, SEQ ID NO:16, ~~SEQ ID N°16~~, SEQ ID NO:17 ~~SEQ ID N°17~~ or SEQ ID NO:18 ~~SEQ ID N°18~~ wherein said method comprises the steps of: --

Please replace the paragraph beginning at page 20, line 14, with the following rewritten paragraph:

D20 -- Preferred isolated recombinant polypeptides of the invention include those having at least 80%, preferably 90%, more preferably 95, and most preferably 98 or 99%, amino-acid identity with polypeptides comprising from amino acid 1 to between amino-acids 1027 and 1145, preferably between amino-acids 1062 and 1145 of SEQ ID NO:20, ~~SEQ ID N°20~~, as well as those having at least 80%, preferably 90%, more preferably 95, and most preferably 98 or 99%, amino-acid identity with polypeptides comprising from amino acid 1 to between amino-acids 984 and 1085, preferably between amino-acids 1019 and 1085 of SEQ ID NO:22. ~~SEQ ID N°22~~. --

Please replace the paragraph beginning at page 20, line 22, with the following rewritten paragraph:

D²¹ -- In a further preferred embodiment, the polypeptide comprises an amino acid sequence having at least 80%, preferably 90%, more preferably 95%, and most preferably 98% or 99% amino acid identity with the amino acid sequence of SEQ ID NO:4, SEQ ID N°4, SEQ ID NO:5, SEQ ID N°5, SEQ ID NO:6, SEQ ID N°6, SEQ ID NO:10, SEQ ID N°10, SEQ ID NO:11, SEQ ID N°11, SEQ ID NO:12, SEQ ID N°12, SEQ ID NO:16, SEQ ID N°16, SEQ ID NO:17, SEQ ID N°17 and SEQ ID NO:18, SEQ ID N°18. --

Please replace the paragraph beginning at page 20, line 30, with the following rewritten paragraph:

D²² -- The invention also relates to secreted soluble $\alpha_2\delta$ -2, $\alpha_2\delta$ -3 or $\alpha_2\delta$ -4 subunit polypeptide comprising amino acid changes ranging from 1, 2, 3, 4, 5, 10, 20, 25, 30, 35, 40 substitutions, additions or deletions of one amino acid as regards to polypeptides of anyone of the amino acid sequences of the present invention. Preferred sequences are those of SEQ ID NO:4, SEQ ID N°4, SEQ ID NO:5, SEQ ID N°5, SEQ ID NO:6, SEQ ID N°6, SEQ ID NO:10, SEQ ID N°10, SEQ ID NO:11, SEQ ID N°11, SEQ ID NO:12, SEQ ID N°12, SEQ ID NO:16, SEQ ID N°16, SEQ ID NO:17, SEQ ID N°17 and SEQ ID NO:18, SEQ ID N°18. --

Please replace the paragraph beginning at page 23, line 5, with the following rewritten paragraph:

D²³ -- **Example 1**

Construction of a nucleotide sequence encoding a soluble secreted human $\alpha_2\delta$ -2 subunit polypeptide deletion mutant of SEQ ID NO:23 N°23

a) Primer design

PCR primers were designed to generate the secreted soluble human $\alpha_2\delta$ -2 deletion mutant of SEQ ID NO:23, SEQ ID N°23 as follows: --

Please replace the paragraph beginning at page 23, line 24, with the following rewritten paragraph:

-- 5' Primer JB197 (5' -TCGCCACCATGGCGGTGCCGGCTC-3', SEQ ID NO:25 SEQ ID N°25)

D24 3' Primer JB198 (5' -TCGGAATTCCTCAGTGATGGTGATGGTGATGGGCCCCGCGGCCACAGTC-3', SEQ ID NO:28 SEQ ID N°28) --

Please replace the paragraph beginning at page 25, line 9, with the following rewritten paragraph:

D25 -- SEQ ID NO:23 SEQ ID N°23 in pFastBac1

Eco RI digest performed on miniprep DNA --

Please replace the paragraph beginning at page 25, line 23, with the following rewritten paragraph:

D26 -- Example 3

Protocol for establishing baculovirus banks for the expression of the $\alpha_2\delta$ -2 deletion mutant SEQ ID NO:23 N°23 --

Please replace the paragraph beginning at page 25, line 29, with the following rewritten paragraph:

D27 -- a) Transposition of DH10Bac *E Coli* cells

One ng (5 μ l) of the recombinant pFastBac-1 construct containing the nucleotide sequence encoding the porcine $\alpha_2\delta$ -2 deletion mutant of SEQ ID NO:23 SEQ ID N°23 was added to 100 μ l of DH10Bac cells thawed on ice. The cells were then mixed gently by tapping the tube then incubated on ice for 30minutes before heat shock treatment by incubation in a 42°C water bath for 45 seconds. The mixture was then chilled on ice for 2 minutes before the addition of 900 μ l of S.O.C. medium. The mixture was then placed in a shaking incubator (200rpm) at 37°C for 4 hours. The cells were then serially diluted (10 fold dilutions from 10⁻¹ to 10⁻³) --

Please replace the paragraph beginning at page 27, line 17, with the following rewritten paragraph:

-- **Example 4**

D²⁸

Expression of the $\alpha_2\delta$ -2 deletion mutant of SEQ ID NO:23 N°23

Please replace the paragraph beginning at page 27, line 27, with the following rewritten paragraph:

-- **Example 5**

D²⁹

Purification of $\alpha_2\delta$ -2 deletion mutant of SEQ ID NO:23 N°23

The $\alpha_2\delta$ -2 ~~The $\alpha_2\delta$ -2~~ deletion mutant of SEQ ID NO:23 ~~SEQ ID N°23~~ was purified from the cell lysate following the purification strategy outlined below: --

Please replace the paragraph beginning at page 28, line 11, with the following rewritten paragraph:

D³⁰
-- The eluate was then loaded onto a Ni-NTA (Qiagen) column (2.5cm i.d. x 6cm h.) pre-equilibrated in 20mM Tris pH8.0, 0.5M KCl, 10mM Imidazole at a flow rate of 2 ml/min. The column was washed successively with buffer A (20mM Tris pH8.0, 0.5M KCl, 20mM Imidazole), buffer B (100mM Tris-HCl pH8.0, 1M KCl), and buffer A again. Elution was performed with buffer C (20mM Tris-HCl pH8.0, 100mM KCl, 0.5M Imidazole). The Ni-NTA eluate (~50ml) was concentrated (30kDa cut-off) to ~2ml and applied at 1ml/min and in 0.2ml aliquots, to an FPLC Superdex-200 column equilibrated in 10mM HEPES, pH7.4, 150mM NaCl. Fractions containing the polypeptide of SEQ ID NO:23 ~~SEQ ID N°23~~ were pulled. --

Please replace the paragraph beginning at page 28, line 21, with the following rewritten paragraph:

-- **Example 6**

D³¹

SPA assay of [³H]gabapentin binding to the secreted soluble human $\alpha_2\delta$ -2
human $\alpha_2\delta$ -2 subunit of SEQ ID NO:23 N°23

Please replace the paragraph beginning at page 29, line 7, with the following rewritten paragraph:

-- Example 7

D³²

Ni Flashplate assay of [³H]gabapentin binding to secreted soluble human $\alpha_2\delta$ -2 subunit of SEQ ID NO:23 N°23

Please replace the paragraph beginning at page 30, line 1, with the following rewritten paragraph:

-- Example 9

D³³

Ni Flashplate assay studying competitive binding of [³H]gabapentin and (S+)-3-isobutyl GABA to human $\alpha_2\delta$ -2-6His (SEQ ID NO:23) SEQ ID N°23)

Please replace the paragraph beginning at page 31, line 1, with the following rewritten paragraph:

-- Example 10

D³⁴

Filter binding assay of [³H]gabapentin binding to the recombinant polypeptide of SEQ ID NO:23 N°23)

Please replace the paragraph beginning at page 31, line 19, with the following rewritten paragraph:

-- Example 11

D³⁵

Construction of a nucleotide sequence encoding a soluble secreted mouse $\alpha_2\delta$ -3 deletion mutant of SEQ ID NO:25 N°25 as follows.

a) Primer design

PCR primers were designed to generate the secreted soluble mouse $\alpha_2\delta$ -3 deletion mutant of SEQ ID NO:25 SEQ ID N° 25 as follows:--

Please replace the paragraph beginning at page 32, line 4, with the following rewritten paragraph:

-- 5' Primer JB201 (5'-TCGCCACCATGGCCGGCCGGGC-3', SEQ ID NO:27 SEQ ID N°27)

D³⁶

D³⁶3' Primer JB202 (5'-TCTCAGTGATGGTGATGGTGATGCGATGCACCCCACACTCTC-3', SEQ IDNO:28 SEQ ID N°28) --